nature OUTLOOK

MEDICAL IMAGING

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Scientific progress is propelled by improved tools of observation. Only with the advent of the telescope could astronomers collect the data that led to theories of planetary motion and gravitation. We are witnessing astonishing improvements in medical imaging, revealing the untold secrets of biology and disease (page S82). This Outlook examines imaging from two perspectives: developments in the technology itself, and applications of imaging to medicine.

One major medical challenge is determining how the brain deteriorates in Alzheimer's disease. Now, imaging is verifying that tangles of tau proteins are leading protagonists (S84). Inflammation is at the core of diverse disorders, from atherosclerosis to cancer, and it is becoming clear that defects in protein clusters known as inflammasomes are primarily to blame. Fluorescent microscopy is starting to show how inflammasomes form and respond to stimuli, and might be worthy therapeutic targets (S86).

Enhanced medical vision makes surgery sharper (S88). Imaging during a breast lumpectomy, for example, allows surgeons to remove the small malignant bits that are often left behind using conventional techniques.

Medical technologists are working on several fronts to improve the quantity and quality of information gathered in a single scan. That often means combining modalities in complementary ways — for example, adding structural information from computed tomography to the functional data from positron emission tomography (S90). And smarter software can focus indistinct blurs (S96). Still, medical imaging is failing to reach its full potential, says Alan Moody. He argues for the creation of "big picture" a network that will enable researchers to readily access the vast library of clinical images that are typically used once and then stashed away (S95).

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Herb Brody
Supplements Editor

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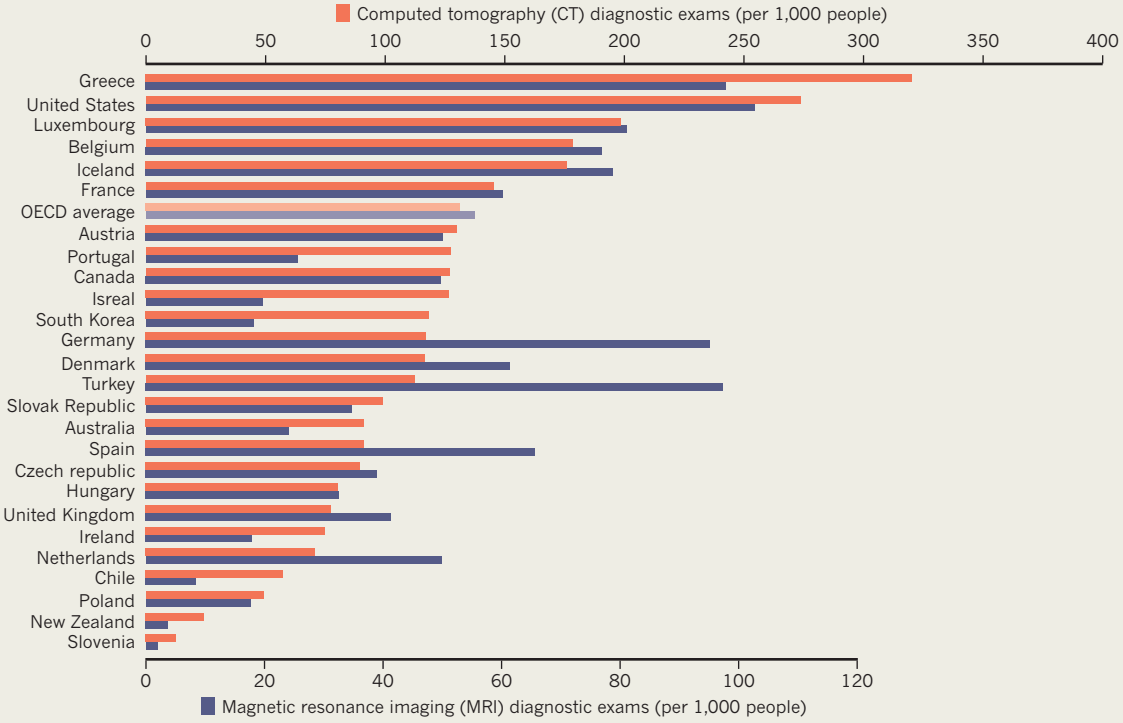
ENHANCED MEDICAL VISION

The ability to look inside the human body without using a scalpel has revolutionized how we diagnose and treat illness and injury. By **Brian Owens**.

UNEVEN DISTRIBUTION

The number of scans per 1,000 people varies widely around the world. In some cases, such as New Zealand, the number may be underestimated because the data only include procedures paid for with public funds.

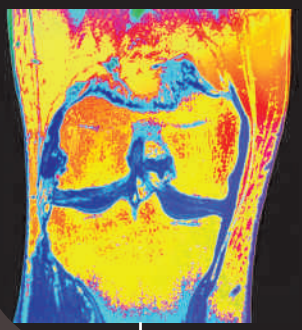
Greece tops the list in number of CT scans because it has a large number of scanners, with the vast majority based in private clinics, and there are no official guidelines governing their use.



TYPES OF SCANS

MRI

Magnetic resonance imaging is used to examine soft tissue such as joint ligaments, muscles, and the brain. It uses powerful magnetic fields to manipulate the spin of protons in the tissue to provide information about how they are arranged.

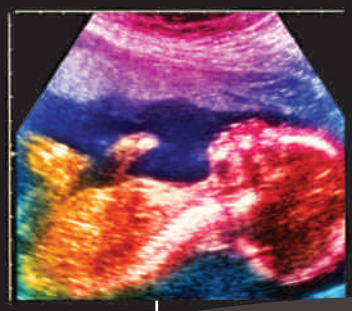


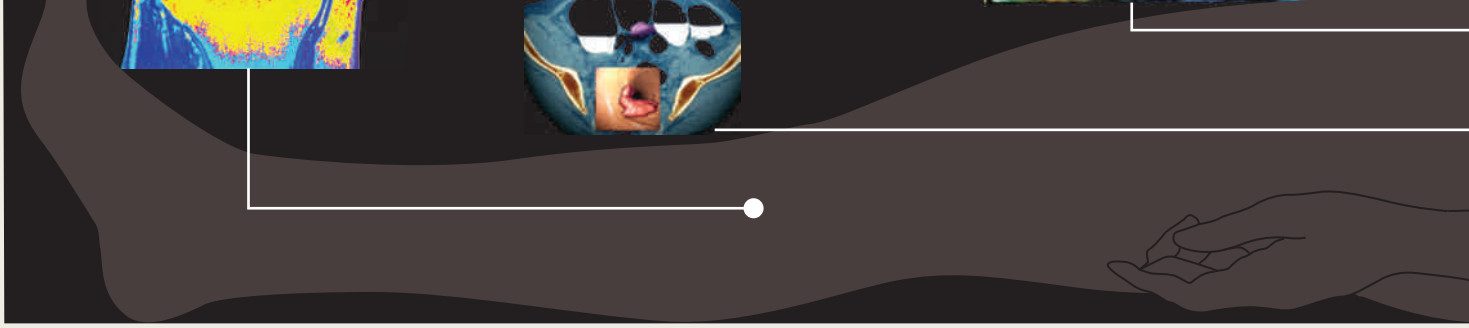
CT

Computed tomography uses computers to process X-rays and create images of slices of the body. These cross-sections can be assembled into larger 3D images to detect tumours, bone damage, and potential hemorrhages, as in this scan of colonic diverticular disease.

ULTRASOUND

Sound pressure waves with a frequency beyond human hearing can be used to image soft tissues in the body, most commonly a developing foetus.

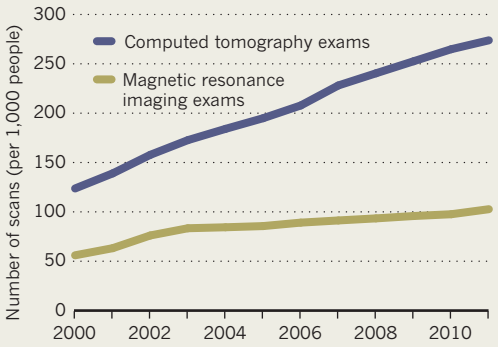




MRI: SIMON FRASER/SCIENCE PHOTO LIBRARY, CT: SOVEREIGN/ISM/ZEPHYR/SCIENCE PHOTO LIBRARY, ULTRASOUND: SCIENCE PHOTO LIBRARY, X-RAY: DEPT. OF CLINICAL RADIOLOGY/SCIENCE PHOTO LIBRARY, PET: PHOTOSTOCK-ISRAEL/CULTURA/SCIENCE PHOTO LIBRARY, FMRI: SOVEREIGN, ISM/SCIENCE PHOTO LIBRARY

RISE OF THE MACHINES

There has been a dramatic rise in the number of CT and MRI scans over the past decade in the United States.

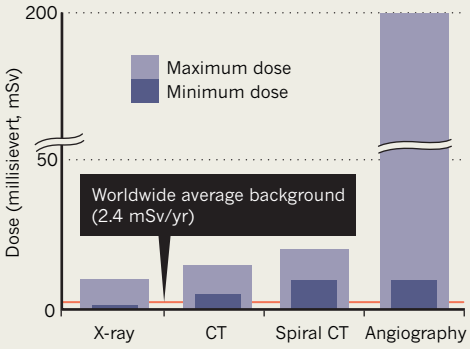


100,000

The most powerful clinical MRI machines have a magnetic field of 3 Tesla, about 100,000 times stronger than the Earth's natural magnetic field

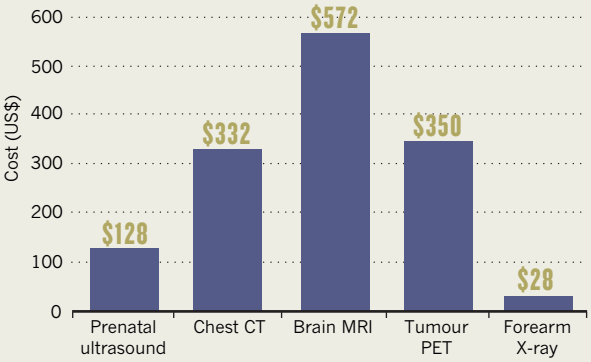
RISKY BUSINESS

Some imaging procedures can expose patients to high levels of radiation, so the number and timing of scans must be carefully controlled over their lifetime.



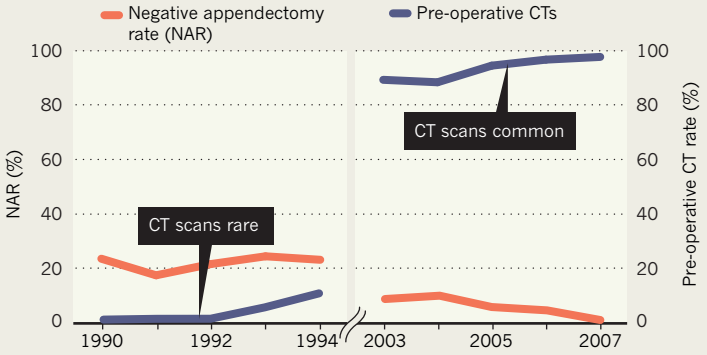
OUT OF POCKET

The cost of a single diagnostic scan can be steep, but can save money in the long run by eliminating the need for expensive surgery (see 'Positive feedback', right).



POSITIVE FEEDBACK

As the use of pre-operative CT to diagnose appendicitis has increased, the number of false positives – and therefore unnecessary surgeries – has dropped to almost none.



X-RAY

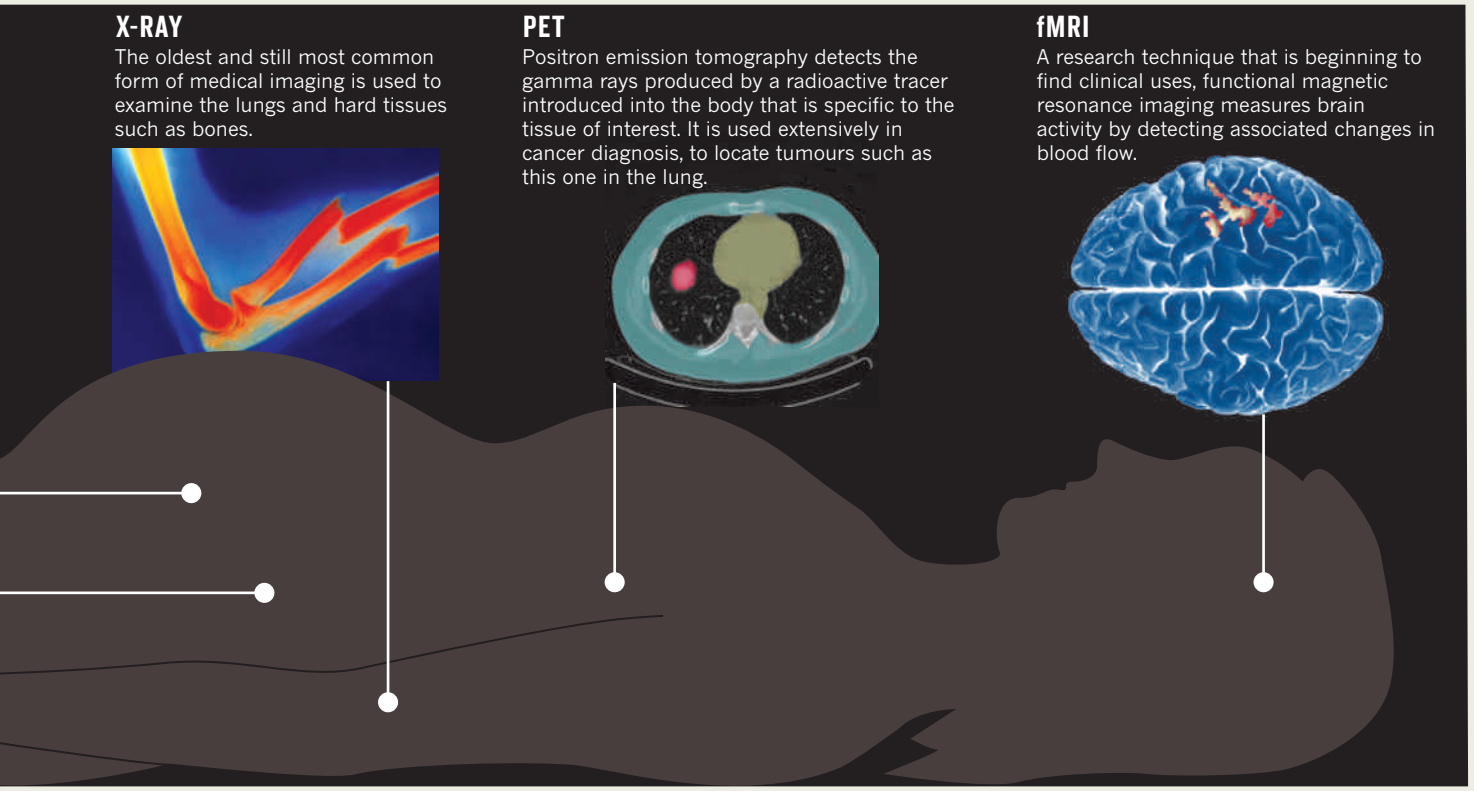
The oldest and still most common form of medical imaging is used to examine the lungs and hard tissues such as bones.

PET

Positron emission tomography detects the gamma rays produced by a radioactive tracer introduced into the body that is specific to the tissue of interest. It is used extensively in cancer diagnosis, to locate tumours such as this one in the lung.

fMRI

A research technique that is beginning to find clinical uses, functional magnetic resonance imaging measures brain activity by detecting associated changes in blood flow.



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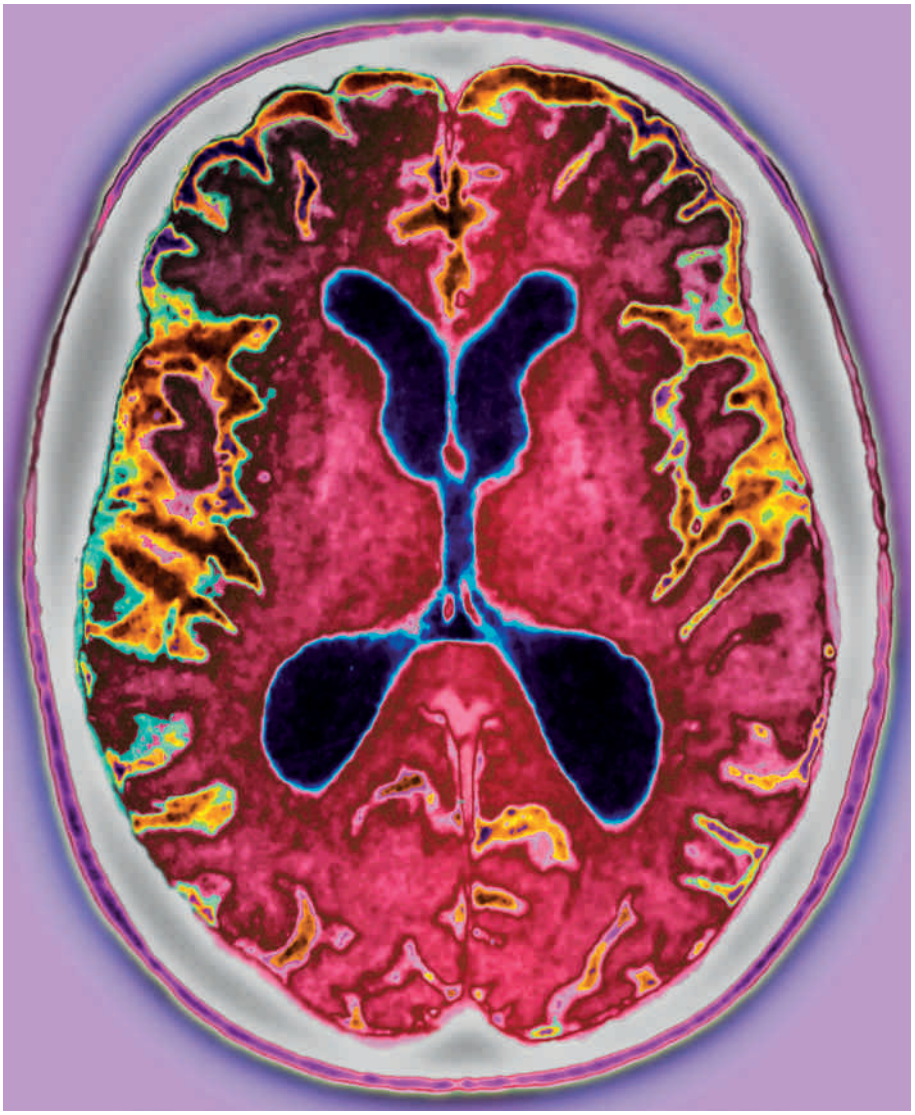


ZEPHYR/SCIENCE PHOTO LIBRARY

Orange and blue indicate deterioration in the brain of a 65-year-old Alzheimer's patient.

ALZHEIMER'S DISEASE

Mapping the brain's decline

Imaging the brains of Alzheimer's patients provides insights into the way this insidious disease progresses.

BY SARAH C. P. WILLIAMS

All the memory and cognition tests indicated that the 87-year-old man had Alzheimer's disease, and his brain scan lit up with yellows and reds. The colours were thought to show the presence of tau tangles, the clumps of proteins in the brain that have been linked with Alzheimer's, but the accuracy of the scan had never been verified in humans. So it was filed away. Two weeks later, the man — a participant in a study by Philadelphia, Pennsylvania-based Avid Radiopharmaceuticals — died from an unrelated cause. When his brain was autopsied, researchers found not only amyloid plaques, giving a definitive diagnosis of Alzheimer's disease, but tau tangles in all the same places that lit up in the scan. For the first time, the accuracy of the scan — a form of *in-vivo* tau imaging — had been confirmed.

Tau imaging isn't the only method being developed to visualize the effects of Alzheimer's disease on the brain, but it's one of the most recent. As these imaging methods improve, researchers hope they will help reveal the underlying causes of Alzheimer's disease, which affects more than 35 million people around the world. Clinicians are hoping the scans will allow them to diagnose patients earlier. And pharmaceutical companies are aiming to develop better drugs, and conduct shorter and smaller clinical trials.

"The point of new imaging is to give you a time-lapse movie of the disease and then see how different interventions change that movie," says neurologist Paul Thompson of the University of California, Los Angeles. "You need to be able to track the spread of the disease in more ways than asking people memory questions."

Scientists know that patients with Alzheimer's disease have a progressive loss of cells in certain areas of the brain, and an increase in two types of protein: amyloid-β, which accumulates to form amyloid plaques; and hyperphosphorylated tau, which forms tangles. But whether tau or amyloid build-ups are causes or effects of the disease is not known. And what other factors are involved remains unclear.

Historically, a tentative diagnosis of Alzheimer's follows a battery of cognitive tests. A definitive diagnosis is only achieved *post mortem* with an autopsy of the brain — a procedure rarely done outside research studies. Since the mid-1990s, clinicians have also been using magnetic resonance imaging (MRI), which shows the loss of brain cells associated with the disease. These scans have shown that the brain begins to change years before symptoms appear.

Nick Fox, a neurologist at University College London, was one of the first to report the changes revealed by MRI up to a decade before the onset of symptoms. For the past ten years he has been involved in a longitudinal study of people with an inherited predisposition for Alzheimer's disease as he explores the early changes to the brain. Comparing MRI scans of individuals over time has allowed him to see which parts of their brain begin to shrink first, and when[1]. "There's a long period of time when people have Alzheimer's disease but typically aren't diagnosed," Fox says.

The MRI scans show that the death of brain cells precedes Alzheimer's symptoms by five or six years. The goal of newer imaging methods is to detect these changes even earlier, and more precisely track disease progression.

BRIGHT SPOTS

Tau imaging uses an experimental form of positron emission tomography (PET). Before the scan, clinicians inject the patient with a tracer molecule called T808 that attaches to any tau protein it encounters — the tracer is what caused the bright spots of yellow and red in the Avid

Radiopharmaceuticals study. But the first PET tracer designed for Alzheimer's disease dates from the early 2000s, when researchers at the University of Pittsburgh unveiled Pittsburgh compound B (PIB), which binds to amyloid[2]. Scientists around the world now use this tracer to track amyloid deposits in clinical trials and research studies. Pharmaceutical companies are looking for amyloid tracers that can be used in imaging studies in the general patient population, not just for research.

The science behind the formation of tau tangles and amyloid plaques is still unclear, however, so not all scientists are convinced that amyloid tracers will be the best way to track the disease. Tau tracers, including T808, are being developed as alternatives, alongside a host of other types of scans. For example, a version of MRI called diffusion tensor imaging can provide detailed images of the connections between parts of the brain and reveal changes to its microstructure. In a study published earlier this year, Fox's team found that such microstructure changes were present in people at high risk of Alzheimer's disease, even when normal MRI could not detect larger structural losses[3].

Other researchers are turning to functional MRI, which shows the areas of the brain that are active during any given task, or when the brain is at rest. They have discovered that a task-free, or resting-state, functional MRI scan reveals alterations in pre-clinical disease, the stage before clinical symptoms[4]. "Even if you only have a basic MRI scanner, you can get a whole handful of these variations of MRI scans in only about 30 minutes," says Thompson.

SEEING RESULTS SOONER

Researchers working to develop scans see their work as a necessary step to improve the way drugs to treat Alzheimer's are studied. "It takes too many participants and too much time to test preventive drugs for Alzheimer's," says Eric Reiman, executive director of the Banner Alzheimer's Institute in Phoenix, Arizona. "We're all interested in developing faster ways to do this."

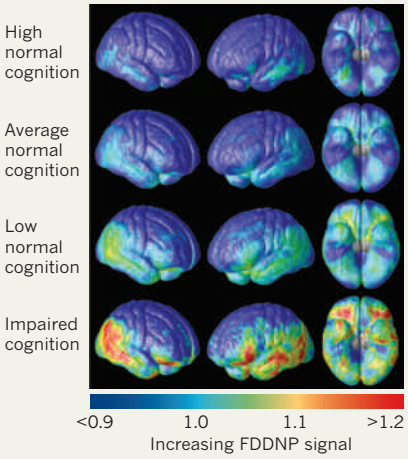
There are many kinds of Alzheimer's disease clinical trials, such as testing drugs or lifestyle interventions, with the aim of preventing disease or treating symptomatic disease. But the primary endpoints are usually the same: measures of cognition, or examinations of autopsied brains. This means that a trial, particularly if it begins before the onset of dementia, can last for decades before reaching an endpoint that shows whether a drug has been successful.

If imaging techniques could provide results sooner, trials could be much less expensive, Reiman says. But the challenge is gathering enough evidence to show that the biomarkers — such as the amyloid or tau PET tracers — are a reliable substitute for measures of actual memory and cognition. "It's a catch-22. You need clinically proven treatments to prove that the biomarkers work," says Reiman. "But the biomarkers will help us develop new treatments much more readily and get them to patients sooner."

One approach, he says, is to focus on populations of people with genetic risk factors for Alzheimer's disease. Testing interventions in such populations requires fewer participants to achieve statistical significance because a higher proportion will develop the disease. So Reiman and his colleagues at Banner have begun working with the US National Institutes of Health and Genentech — a biotech subsidiary of F. Hoffmann-La Roche based in South San Francisco, California — to test an amyloid antibody called crenezumab in an extended family in Colombia that has a genetic mutation leading to early onset Alzheimer's. As well as testing the drug, the scientists are using PET and MRI scans with the latest tracers to track the disease in the participants. They have detected amyloid plaques in family members from around 28 years of age, almost two decades before the typical onset of disease in those without the mutation.

SLIPPING AWAY

Positron emission tomography scans with a tracer (FDDNP) that binds to both amyloid plaques and tau tangles visibly correlate with cognitive decline.



PAUL THOMPSON/UCLA.

GENES AND SCREENS

The latest forms of brain imaging, which provide increased precision and earlier glimpses of disease, along with the decreasing costs of well-established scans, are a boon to pharmaceutical companies looking to test drugs. But they also offer basic researchers a way to find out what causes Alzheimer's.

At UCLA, Thompson heads Project ENIGMA, the world's largest brain imaging study. Scientists based in 20 countries contribute all types of scans of their patients' brains, along with information on the patients' health and genetics. The network, which so far includes more than 26,000 brain images, provides a way to do large-scale automated studies on the specific features of brains from people with Alzheimer's disease or with genetic risk factors for Alzheimer's. "When we pair imaging and genetics, we can screen the brains of certain gene carriers and compare them with non-carriers," Thompson explains. "Then we can ask: what's different about these brains?"

When they looked at one gene known to be a risk factor for Alzheimer's, *CLU*, the ENIGMA researchers discovered that a variant of the gene can damage wiring in the brain when a person is about 20 years old[5]. Thompson says that the variant of *CLU*, which encodes the protein clusterin, "doesn't actually give you Alzheimer's directly, but it gives your brain a punch that makes any second blow harder to deal with."

More recently, researchers found another risk gene for Alzheimer's, *TREM2*, that is prevalent in Iceland[6]. Within five months, Thompson's team had sorted through the ENIGMA scans to find entries that carried the gene variant. They were able to show, in an as yet unpublished study, that the *TREM2* variant speeds up brain cell loss once someone has developed Alzheimer's disease.

As such discoveries progress — linking genetics to structural and functional changes in the brain using automated, high-throughput methods — Thompson hopes that some common molecular pathways will emerge that can help explain dementia. By seeing inside the brain, the scientists can get a clearer picture of what happens during the onset and progression of Alzheimer's.

These latest brain scans are now widely used for Alzheimer's clinical trials and research studies, but they are far from routine in the clinic. A definitive, early diagnosis of the disease can give patients and their caregivers a better idea of the prognosis, but there isn't enough evidence that the results of a scan will change the clinical outcome. It will take an ongoing interplay between better imaging and the development of treatments to change this, says radiologist Clifford Jack, an Alzheimer's imaging specialist at the Mayo Clinic in Rochester, Minnesota. Both sides of the equation — scans with the ability to screen disease, and treatments that slow preclinical disease — will need to be developed first. "At some point, we will develop screening methods and early intervention treatments," says Jack. "Just like with cardiovascular disease today, we can identify people with high blood pressure and high cholesterol, which may not be provoking symptoms, and intervene." Until then, improvements in brain imaging techniques will help scientists working on Alzheimer's disease to better understand this devastating and deadly cognitive decline. ■

Sarah C. P. Williams *is a freelance science writer based in Kailua, Hawaii.*

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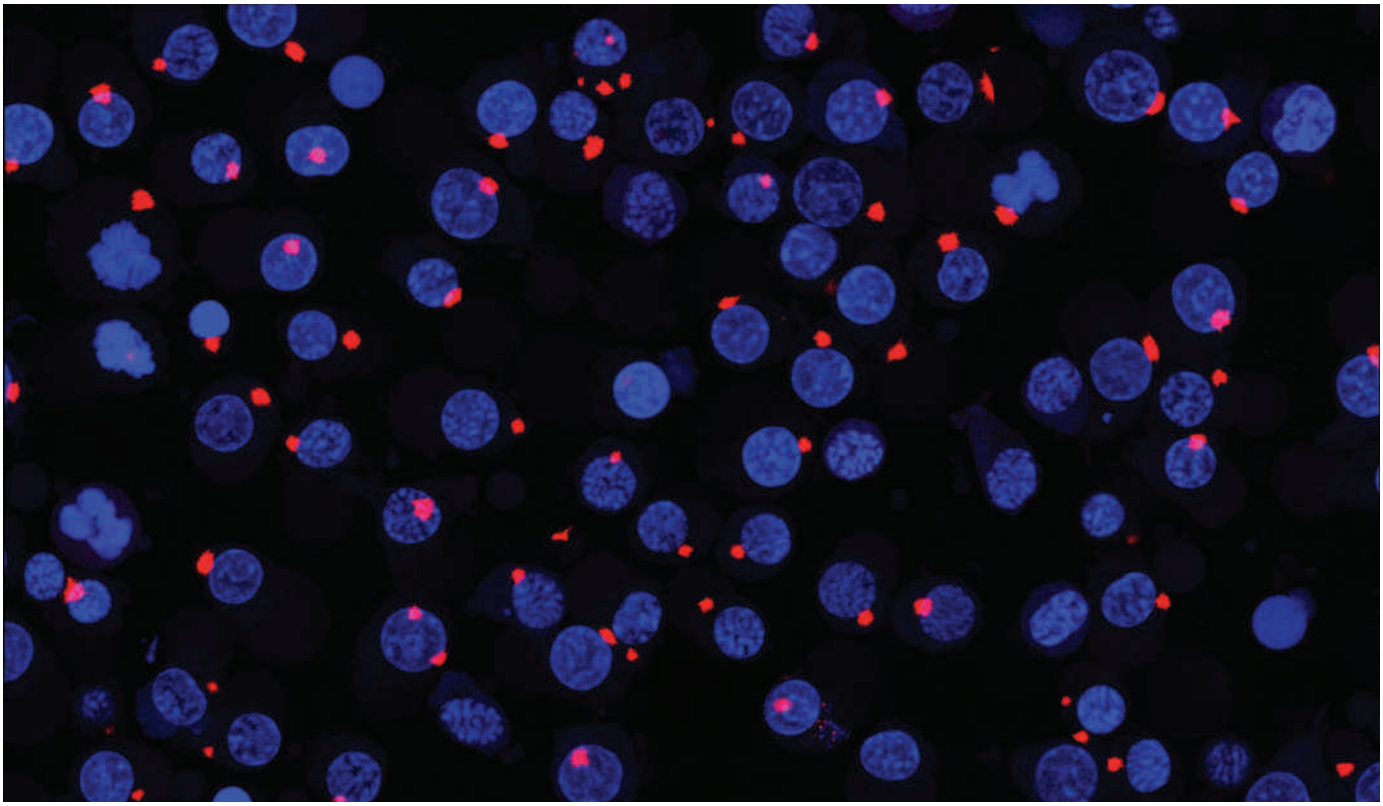

EICKE LATZ

One burning question: why is there only one inflammasome (red dot) per cell (nuclei stained blue)?

INFLAMMATION

A complex problem

Multi-protein inflammasomes are being implicated in a surprising number of diseases, and researchers are keen to find out why.

BY KATHARINE GAMMON

The immune system's primary task — telling friend from foe — is no easy job. Faced with a serious threat such as an infection or wound, the system needs to send defenders out immediately. But it also needs to lie low in the face of innocuous visitors.

When confronted by a dangerous outsider, the human body has two lines of defence. There is learned immunity, which is acquired by exposure to a pathogen, either from the environment or through a vaccine. Then there is innate immunity, which is the immediate hard-wired reaction to outside invaders. The key ingredients in the innate response are inflammasomes — large protein complexes that form in response to a perceived threat, sounding the alarm for the body's inflammatory responses.

A growing body of research indicates that defects in the structure and activity of inflammasomes are central to a vast number of illnesses, from atherosclerosis and arthritis to Crohn's disease, cancer, diabetes and irritable bowel disease. Targeting these complexes could usher in a new age in drug development. But doing so requires precision methods of seeing inflammasomes at work.

SOUNDING THE ALARMIN

Over the past decade, researchers have begun to piece together how inflammasomes work. These large molecular complexes form in response to different stimuli — and their composition can differ accordingly. The stimuli can be incredibly diverse, including bacteria and bacterial toxins but also other 'danger signals' such as cholesterol crystals. Once they have formed, they activate the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18, which then call in lymphocytes to battle the perceived infection.

Scientists once believed that the only job of the innate immune system was to protect the host from external danger. Recently, however, a more nuanced understanding has emerged. It is now clear that the innate immune system — and inflammasomes in particular — can be activated by endogenous molecules known as alarmins, released by damaged cells, or danger-associated molecular patterns. Cholesterol crystals are one example; others are monosodium urate crystals in gout, cholesterol crystals in atherosclerosis, islet amyloid polypeptide, which is deposited in the pancreas in type 2 diabetes, and even simple molecules such as ATP, which is released from damaged or dying cells.

The picture is getting murkier. "Finding that the innate immune system can actually be activated by self-molecules has challenged the traditional view that the immune system only recognizes non-self as harmful," says Kate Schroder, a molecular cell biologist at the University of Queensland in Brisbane, Australia.

UNDER THE MICROSCOPE

Researchers are starting to probe the role of inflammasomes in providing defence and understanding how they distinguish harmful from harmless. One of the ways they are

building up detail is through creating images of the complex assemblages using standard confocal microscopes combined with fluorescent tagging. Confocal microscopy uses mirrors to focus light on a sample at multiple points and depths, building up a three-dimensional image. When fluorescent molecules are attached to proteins of interest — such as those that make up the inflammasome — they show up inside the cell samples.

Such fluorescent imaging is at the heart of a study published in August 2013 linking Alzheimer's disease to inflammation-causing proteins[1]. In it, researchers showed that mice lacking a molecule necessary for the activation of an inflammasome called NLRP3 did not develop the disease — suggesting a potential target for future treatments. "When you do not have inflammasomes, you do not develop any Alzheimer's, which is really cool," says Eicke Latz, one of the study's authors and director of the University of Bonn's Institute for Innate Immunity in Germany. "So you could imagine that blocking this pathway could be beneficial in humans."

Latz has also used a combination of laser reflection and fluorescence confocal microscopy to identify the way that crystalline materials interact with immune cells. His team found that feeding mice a high-fat diet caused small cholesterol crystals to appear in as little as two weeks. High levels of these crystals embedded in the vascular wall then led to activation of IL-1β (ref. 2). The researchers still do not know how the crystals activate the inflammasome, but they did identify that the complex is the trigger for the escalating response.

The emerging connection between cholesterol crystals, IL-1β and atherosclerosis — which is an inflammatory reaction that takes place in fatty blood vessel walls has prompted the first clinical trial of a drug to block IL-1β. In CANTOS, a double-blind, placebo-controlled trial run by Swiss company Novartis, 17,200 men and women who have had heart attacks are being treated with canakinumaba human monoclonal antibody that neutralizes IL-1β. The trial will follow the patients for four years and monitor whether they have any further cardiac events and other health outcomes.

Another approach looks at inflammasomes in macrophages, which are scavenger cells that consume dead cells, bacteria and viruses. Denise Monack, a microbiologist at Stanford University in California, is using confocal microscopy along with a technique called array tomography to image macrophage inflammasomes responding to bacteria such as *Salmonella* or *Fransicella*, as well as searching for genes that influence inflammasome activation.

In array tomography, Monack's group creates thin serial sections of a sample of cultured cells and stains them with fluorescent antibodies. The technique was developed by Monack's colleague Steven Smith in 2007, and Monack's lab is the only one to use it to image inflammasomes. Millions of images are snapped with a microscope and assembled into a three-dimensional structure using software. The cells can be stained with special substances packed with electrons, allowing researchers to take images at higher resolution with an electron microscope.

Imaging macrophages serially — creating two-dimensional image tiles that are reconstructed computationally into three-dimensional images — increases the resolution of the sample by an order of magnitude (from 700 nanometres down to about 70). Monack says that array tomography combines features of modern optical fluorescence and electron microscopy with better spatial resolution than confocal imaging[3], making the technique ideal for imaging the molecular architecture of an inflammasome.

"We can't explain why, but inflammasomes seem to be playing a role in so many diseases."

Monack has found that not all macrophages form inflammasomes, and that when there is an inflammasome there is only one per cell. "That's one of my burning questions: why is there only one inflammasome?" The answer, she surmises, may be that there is some kind of anchoring platform structure in the cell that allows for only one complex to form.

THE REAL THING

Dissecting this type of detail, using techniques such as array tomography or confocal microscopy, could help identify potential treatments for a range of inflammation-related conditions. "By examining the [inflammasome] structure, we may be able to design small molecule inhibitors to block either the formation of the enzymatic complex or the processing of the cytokines," says Ashley Mansell, head of the Toll-Like Receptor (TLR) Signalling Laboratory in the Centre for Innate Immunity and Infectious Diseases at the Monash Institute of Medical Research in Melbourne, Australia. One such application might be influenza: Mansell is interested in why pandemic strains drive hyper-inflammatory responses, whereas regular influenza strains do not[4].

Influenza is not the only point of interest — researchers are probing other, chronic illnesses to find targets for inflammasome inhibition. Latz points to preclinical studies that show that mice treated with an IL-1 antibody do not develop diabetes. His team is using high-throughput screening to look for other inflammasome inhibitors, but Latz thinks it will take a while to develop such a drug for clinical use. "A real inflammasome inhibitor would be nontoxic, specific and have no side effects — and that hasn't been established," he says, although he adds that the field is so new that more advances will come in the next few years.

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How are inflammasomes activated and regulated:
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A deeper understanding of inflammasomes could also be useful for diagnostics. Mansell is working on ways to get an earlier and better diagnosis of atherosclerosis or cholesterol problems with a blood test, searching for markers of inflammasome activation in the serum.

Mansell says one potential scenario is a doctor testing a patient's blood for IL-1β, and correlating that with other risk factors to offer an early warning sign. "Inflammasome activation is a marker that means there is a disruption of homeostasis," he says.

Treating or halting inflammasome activation early could stop a disease in its tracks. Mansell points to hypertension, which is a precursor to many illnesses from stroke to heart disease. Recent studies indicate a relationship between hypertension and inflammasome activation. If this link is proven, and a test developed, "we could offer people a way to treat or stop the disease from progressing further", he says.

Pharmaceutical companies are already investigating inflammasome-based therapeutics. For example, Idera Pharmaceuticals, based in Cambridge, Massachusetts, is working on treatments for psoriasis, lupus and arthritis that block toll-like receptors, which mediate the inflammasome response. And Navidea Biopharmaceuticals, based in Dublin, Ohio, has recently received approval for Lymphoseek, a lymphatic mapping agent that binds to a receptor known as CD206 on the surface of macrophages and dendritic cells — both of which are immune cells that house inflammasomes. The radiopharmaceutical binding agent allows doctors to see lymph nodes in the potential drainage path of a tumour. Originally designed for the lymph nodes around breast cancer, the agent could also point to early inflammasome involvement in some 15 diseases, says Fred Cope, Navidea's chief scientific officer.

Mansell says that this embryonic field has extensive options, because inflammasome activation is implicated in such a wide swathe of maladies. "Inflammasomes are the sparks that set things off — we can't explain why, but they seem to be playing a role in so many diseases," he says. "There have been a number of real breakthroughs in this area over the past few years." Bringing inflammasomes further into focus should yield many more. ■

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DR DANIEL RUAN AND DR JAYENDER JAGADEESAN

Imaging tools act as an extra pair of eyes for the surgeon.

SURGERY

The eyes of the operation

Real-time imaging of a patient's body is guiding surgeons and radiologists past healthy tissue to the diseased cells.

BY JESSICA WRIGHT

In 1988, when David Jaffray was a summer student, he watched as a team of surgeons prepared to treat a child with brain cancer, aiming carefully planned radiation treatment at a fuzzy X-ray image.

"I thought to myself, a huge amount of effort has gone into designing the treatment for this child, and we have all this technology and all these people standing around trying to get this right, and we can't see what we're doing," says Jaffray, a medical physicist at the Princess Margaret Cancer Centre in Toronto, Canada. He has worked ever since to engineer solutions to this problem.

Jaffray's experience shows that in many ways, doctors work in the dark. A suspicious lump of cancer looks exactly like the rest of the breast, for example. The prostate can hide a tumour that can evade multiple biopsies. But what the human eye cannot see, precision medical imaging can — whether it's magnetic resonance imaging (MRI) or computed tomography (CT), for example. For the past twenty years, researchers have worked to turn these scans into maps that reveal the hidden insides of a patient's body, guiding surgery or radiation therapy.

In 2003, Jaffray helped design the first radiation machine with a built-in CT scanner. This equipment allows radiologists to trace the outline of the tumour before each dose. The resulting confidence that they can avoid healthy tissue has allowed doctors to increase the dose of radiation. In the brain, imaging reveals not just the structural outlines of a tumour, but which parts can be cut out without dire consequences. And researchers are working to adjust these maps during surgery, so they update in real-time, moving and shifting along with the patient.

TUMOUR TOPOLOGY

Image guidance is particularly valuable for cancer treatment because it addresses the primary challenge: how to remove every last bit of a tumour while damaging as little healthy tissue as possible. Imaging during a breast cancer lumpectomy, for example, allows surgeons to remove the small 'breadcrumbs' of cancer that are often left behind, significantly reducing the risk of recurrence, says radiologist Ferenc Jolesz, director of the National Center for Image-Guided Therapy at Brigham and Women's Hospital in Boston, Massachusetts.

In the early 1990s, Jolesz pioneered the use of MRI in operations, taking scans during brain surgery for the first time. When this was successful, it became clear that the best way to guide treatment would be to combine as many forms of imaging as possible, says Jolesz. In September 2011, a grant from the US National Institutes of Health led to the Advanced Multimodality Image Guided Operating (AMIGO) suite — a three-room operating suite that includes an MRI scanner, a CT and positron emission tomography (PET) scanner, and an advanced three-dimensional ultrasound and navigation system.

Researchers are exploring how to combine the resources at AMIGO to refine treatments. Imaging during surgery can address the problem of overtreating early-stage tumours, such as those found during routine lung CT scans on smokers. Small lumps are difficult to locate so surgeons may end up removing large pieces of lung tissue that will never grow back, says Raphael Bueno, a thoracic surgeon at Brigham and Women's Hospital. As part of an ongoing clinical trial, Bueno has devised a method to use a CT scan to guide the placement of a small

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Two-photon microscopy tracks B cells in the spleen:
go.nature.com/yrfcdb

hook-like device in the lesion. The hook is attached to surgical thread that reaches out of the lung. During surgery the thread acts as a guide, allowing Bueno to snip out only the affected tissue.

But nowhere is accurate targeting more essential than in the brain, where the neurons that control key functions may snake past a tumour or through the target site of epilepsy surgery. What's more, a tumour can reorganize the brain's function, shifting the neuronal connections. To address this, surgeons are developing ways to glean information from MRI scans — such as functional MRI (fMRI) or diffusion tensor imaging (DTI) — that map not only the brain's structure, but also its function.

Functional MRI highlights the parts of the brain that receive the most blood when patients perform a task, revealing which regions may be involved in certain functions. And DTI analyses the diffusion of water molecules in the brain that orient in the same direction as the long tracts of neurons. Researchers can turn these data into maps that paint brain tracts different colours according to their direction, identifying information highways once invisible to surgeons.

Using these scans to inform surgery has dramatically improved patients' outcomes, says Christopher Nimsky, a neurosurgeon at the University of Marburg in Germany. "Ten or twenty years ago we accepted that we would cause 10% or 15% new neurological deficits after surgery." Thanks largely to the use of imaging during surgery, he says, that figure is "now down to 2% or 3%, even in the complicated cases."

The functional maps, which doctors have begun to use routinely in the past five years, help them decide which patients would be too impaired by surgery to make it worthwhile. But they also give doctors the confidence to pursue more aggressive surgery in certain cases. Using DTI may reveal that although a tumour is in a functional region, it sits further away from essential neuronal connections than the surgeon originally thought, allowing them to remove more of the tumour or surrounding region.

These technologies were originally designed as research tools. Turning the data into maps that report on the location of important brain regions is complex and in many ways subjective. "The challenge is cutting to the core of what information the surgeon really needs," says Alexandra Golby, a neurosurgeon and researcher at Harvard Medical School in Boston, Massachusetts. "The clinician wants to ask, is that tract behind the tumour or running [through] the tumour? Which tracts am I likely to encounter on my way in? Is this area connected to that area?"

Many surgeons now routinely use Slicer, an open source software tool introduced in 1999 that combines structural MRI scans with fMRI and DTI data in three dimensions[1]. Shown all at once, the tracts produced by DTI analysis look "like a bowl of spaghetti", says Golby. In 2011, Golby added a module to Slicer that allows surgeons to isolate any of the tracts that pass through a boundary they have defined around the tumour. Surgeons can also highlight a particular spot in the brain, and view only those tracts that pass through it. During the operation, these maps may be combined with the surgical navigation guidance system, which uses structural data to show surgeons where they are in the brain.

One crucial feature is that the outlines traced on imaging scans accurately reflect the boundaries of the tumour. In fact, different scans, such as CT and MRI, may not even match each other, says Kristy Brock, a medical physicist at the University of Michigan in Ann Arbor. It's fascinating to see how different the tumours look on all these things," she says.

To get a better understanding of these outlines, Brock is working alongside radiologists, oncologists, surgeons and pathologists in a new field called correlative pathology. In one study, Brock and her colleagues took images of the liver before and after it was surgically removed. They then sliced it into sections, photographing each one to create a three-dimensional picture. Finally, they used histology to stain cancer cells a different colour from the rest of the tissue so they could identify the cancer at the cellular level in each slice[2]. By combining these layers of information, researchers can get closer to understanding how accurately MRI defines the boundaries of a tumour, says Brock. They can also compare different imaging technologies to determine which scan, or combination of scans, most accurately represents reality.

MAPPING IN REAL TIME

For even more precision, however, an imaging system should take into account the significant changes that occur during surgery. Once the skull is open, cerebrospinal fluid leaks out and the brain bulges from the skull. And as the surgeon cuts into the tissue, there are even more changes. With an MRI machine in the operating theatre, doctors can match the tract reconstructions with the shifting reality of a brain in an open skull.

The challenge is to take the surgical scan as quickly as possible and line it up accurately with the pre-surgical images, says Gavin Winston, a neurologist at University College London. Winston is aiming to do this for the optic radiation, a vision tract that often passes through the site targeted for epilepsy surgery. He has developed computer adjustments that allow doctors to take a second DTI scan during surgery and line this up with the existing DTI tracts in a matter of minutes[3].

This innovation can have real benefits for the patient's quality of life. Many patients opt for epilepsy surgery with the hope of qualifying for a driving licence. But damage to the optic radiation can impair their vision so much that they still can't drive, Winston says. His team has used this technique on 12 patients so far, and all 12 were able to drive after surgery.

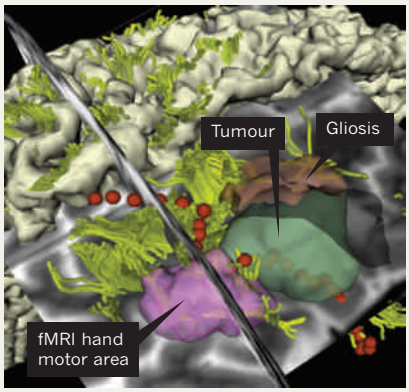
"It's fascinating to see how different the tumours look on all these things."

But taking an MRI or DTI scan during surgery is extremely difficult, says Golby. "You have to stop, get the metal out of the way, and then move the patient into the scanner, or move the scanner over the patient," she says. "We've come to take it for granted that we can get these gorgeous pictures of the insides of people's bodies, but it takes a huge technical tour de force to do so." Golby is developing simpler methods to keep surgeons updated. One option is to use ultrasound, which is easy to use and cheap. Ultrasound provides much sparser data than MRI, she says, but may be sufficient to tell the surgeon when something significant has changed in the brain.

All these approaches allow researchers to consider each patient's biology and to base treatment on their unique response. "The idea that treatments can be more precise by integrating imaging is sort of obvious," says Jaffray. "But the technological advance that allows us to do that in a non-invasive way is pretty remarkable," he adds, considering how much the tumour changes shape over time. He envisages "fleets of multimodal treatment machines" that can make these adjustments consistently — systems that will, in effect, bring superhuman vision to those seeking to repair the human body. ■

LOCATE AND DESTROY

A 3D reconstruction using data from several imaging technologies reveals a tumour.



ALEXANDRA GOLBY, SONIA PUJOL, AND ISAIAH NORTON, BRIGHAM AND WOMEN'S HOSPITAL

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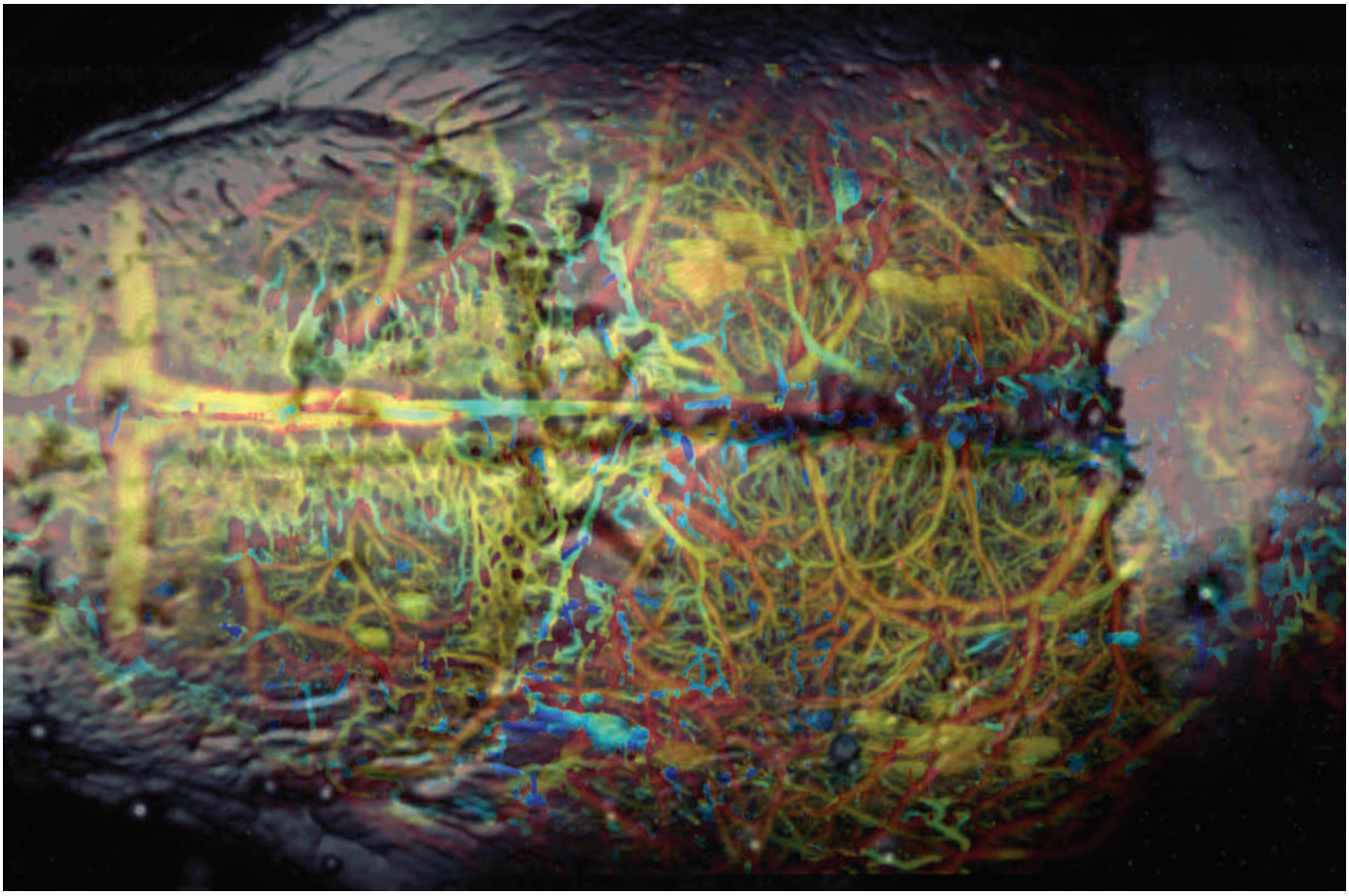

JUNJIE YAO AND LIHONG WANG

Photoacoustic microscopy reveals the microvasculature of a mouse brain, while ultrasonic microscopy gives a glimpse of the skull (grey).

TECHNOLOGY

Multiple exposure

Combining imaging techniques can provide a wealth of information about disease.

BY NEIL SAVAGE

In 1991, David Townsend, a physicist then at the University of Geneva in Switzerland, built a low-cost positron emission tomography (PET) scanner. The design left some spaces in the instrument's structure, and Townsend wondered whether he could fill them, and improve the machine, by squeezing a second scanning technology into the gaps. A doctor friend told him that surgeons were more familiar with the anatomical information provided by computed tomography (CT), so he added that, and the PET–CT scanner was born.

At first, many in the medical establishment were sceptical about the instrument's potential. "They all thought David Townsend was kind of loony," says Michael Vannier, a radiologist at the University of Chicago. "There was a lot of foot dragging associated with PET–CT."

But what was unfamiliar 15 years ago has since become the norm. These days, "any sensible person would not use PET alone," says Vannier, adding that the PET–CT scanner has "vastly improved doctors' ability to identify the stage of a lymphoma, allowing more nuanced treatment decisions." Simon Cherry, a biomedical engineer at the University of California, Davis, agrees. "It's almost impossible to buy a PET scanner without a CT scanner attached to it now," he says.

That success has spurred other efforts to combine imaging modalities. None of the many ways of looking inside the human body is perfect, but merging the strengths of two or more technologies may allow physicians to see details they have never seen before and improve the detection, diagnosis and treatment of ailments ranging from cancer to heart disease or Parkinson's disease.

Imaging techniques mainly show either structural information — the physical shape of an organ or a tumour — or functional information, such as which molecules are present or what metabolic activity is occurring. But no single technique is optimal for both. Coronary angiography, for example, in which X-rays image a contrast agent flowing through the heart's blood vessels, provides "exquisite detail" of those vessels, but doesn't show whether the cells in the heart muscle are living or dead, says Vannier. If they're dead, coronary bypass surgery would be ineffective. "You could improve the circulation," Vannier says, "but the heart wouldn't beat any better."

Similarly, X-ray tomography can spot tiny breast tumours but has trouble telling which are benign and which are malignant. This lack of functional information leads to a lot of false positives, followed by unnecessary surgery, says Lihong Wang, a biomedical engineer at Washington University in St Louis. "If you can tell the function, you can get more information," Wang explains. "The end result is higher accuracy."

Wang is trying to improve endoscopy by combining conventional ultrasound with a technology called photoacoustics. Ultrasound

endoscopy provides high-resolution images of structures and is widely used to look for oesophageal or colorectal cancer, for example. But its contrast is low, so it does not readily distinguish between blood vessels and lymphatic vessels, or healthy and diseased soft tissue.

Photoacoustic tomography fills that gap by helping to identify different types of molecules[1]. To obtain a photoacoustic image, Wang delivers pulses of light through an optical fibre to the tissue being examined. When the tissue absorbs the light, it heats slightly and expands, sending out a pressure wave that can be detected by the same sort of receivers that pick up the ultrasound signal. Different substances in the body — water, lipids, melanin — absorb different wavelengths of light, so by selecting the wavelengths, investigators can identify what they're looking at. Two particular wavelengths of visible light, 562 nm and 584 nm, can distinguish between haemoglobin that's saturated with oxygen and haemoglobin that lacks oxygen. This can reveal a tissue's rate of oxygen consumption, which is a measure of metabolic function. Near-infrared light with a wavelength of about 1,200 nm can pick out lipids and allow doctors to tell if a plaque inside an artery is vulnerable to breaking off and causing a clot. Wang suggests that the absorption of photons by DNA could allow photoacoustic imaging of cell nuclei, making it possible to perform a sort of on-site biopsy without removing any tissue.

Both photoacoustic and ultrasound imaging systems use an ultrasonic transducer to process the signals, so they can easily be incorporated in the same machine, Wang says. He is currently working with Philips Healthcare to build a commercial version.

The structural information provided by ultrasound can also be combined with the molecular information available through confocal microscopy, an optical technique that provides high-resolution images, allowing scientists to identify molecules when a fluorescent tag latches onto them. "They're perfectly complementary techniques," says Christopher Contag, a microbiologist at Stanford University's Molecular Biophotonics and Imaging Laboratory. He suggests going even further in combining techniques: adding a third, such as photoacoustics, would make the images even richer. "Every modality gives you a little bit different information, so a combination of them gives you some varieties to choose from," he says.

Contag's approach could provide enough information for doctors to perform histopathology inside a living patient, instead of on a piece of excised tissue in a lab. This would not only reduce the need for biopsies, but could also improve the accuracy of the diagnosis. Tissue that has been removed and stuck to a microscope slide might be dried out, reshaped or otherwise changed by the process. Leaving it in place avoids those problems.

Combining modalities in this way could also extend the use of molecular probes. Using dyes already approved by the US Food and Drug Administration to stain tissue inside a patient could make Contag's vision of *in vivo* biopsy possible — and new molecular probes might make it possible to detect cancers at an earlier stage. One probe, developed by Stanford's Matthew Bogyo, tests for legumain, a protease produced by most cancers in their early stages. Such a probe would make it easy to spot the early signs of cancers in the microscope, Contag says. "If there's a tumour, it's activated and it lights up like a beacon."

COMPARING CONTRASTS

Another approach to making images richer is to mix together not data from different technologies, but different results from the same technology. Multispectral magnetic resonance imaging (MRI) is a case in point. Conventional MRI uses radio-frequency pulses and changes in the magnetic field to create an image. Each specific sequence of pulses provides its own type of image, or contrast, making different types of tissue easier to see.

A group led by neuroscientist Suzanne Corkin of the Massachusetts Institute of Technology combined four of these contrasts to look for early signs of the brain damage caused by Parkinson's disease[2]. They wanted to measure the volume of a brain region called the substantia nigra, which is known to deteriorate in Parkinson's. Post-mortem examinations of the brains of Parkinson's patients have led to a theory of how the damage progresses, but doctors had been unable to see the changes in a living brain.

"We can start going deeper and deeper."

Two of the contrasts provided by different pulse sequences show the boundaries of the substantia nigra, but individually each is a bit fuzzy, says David Ziegler, a neurologist at the University of California, San Francisco, who worked with Corkin on the MIT project. A third contrast shows white matter appearing very bright, but cannot distinguish it from cerebrospinal fluid, whereas a fourth makes the fluid look black, so putting those two contrasts together makes it clear which is which. The team took images of volunteers using the four contrast methods in sequence before combining them into a single image. "Now the boundaries of the substantia nigra and the red nucleus [another brain structure] just sort of pop," Ziegler says. Although such a small study (29 Parkinson's patients and 27 controls) could not provide definitive results, the images seemed to confirm what the post-mortem data had suggested about the way the disease progressed.

Multispectral MRI could lead to the earlier detection of Parkinson's, and perhaps earlier treatment, and might provide a greater understanding of the mechanisms of the disease. "We can start going deeper and deeper to detect the earliest losses that might be associated with Parkinson's," Ziegler says. They might learn even more by marrying that ability to PET scans or electroencephalograms.

The trick to multispectral MRI is to take all the scans in the same session. Any MRI image has some distortions, which can be corrected for. But with multiple scans, the images must be aligned correctly for that to work; scans from separate sessions would be hard to line up. "You can't take differently distorted images and combine them in any meaningful way," Ziegler says. The MIT team scanned the patients with the four contrast methods back to back, then immediately repeated the sequence, in a process lasting about an hour. They then averaged the two sets to correct for artefacts from movement, and then combined the four contrasts into one image.

CLEAR GUIDANCE

The latest combined technology is the PET–MRI scanner, designed by Cherry and only just coming onto the market. Many major US hospitals don't yet own one. Using PET, which is useful for finding biomarkers, adds functional information to the MRI image. The combination could improve imaging of the brain and of the soft tissue in the pelvic area, helping to identify such diseases as bladder or brain cancer. Cherry says the combination provides the best of both worlds: "PET is the most sensitive technology out there. MRI is the highest-contrast technology."

At roughly US$5 million per machine (roughly double the cost of high-end MRI), PET–MRI faces a high hurdle to widespread adoption. And some scientists are sceptical. "We don't yet have examples where PET–MRI is clearly superior to PET–CT," Cherry admits.

But Vannier is afraid that being too cautious in developing new combinations of imaging techniques could hobble medical progress. As medicine gets better at detecting more cancers and other abnormalities, he says, it's important to distinguish those that are potentially lethal from those that are unlikely to become life threatening. And better identification of vulnerable plaques could revolutionize coronary care and minimize unnecessary treatments that can do more harm than good. "The ultimate goal is to not over-treat or do one size fits all," he says.

Imaging that combines structural and functional information might not only increase early detection, but also guide doctors about what to treat and what to leave alone. "The imaging," Vannier says, "will provide the tool that in clinical practice will allow you to confidently make the decision." ■

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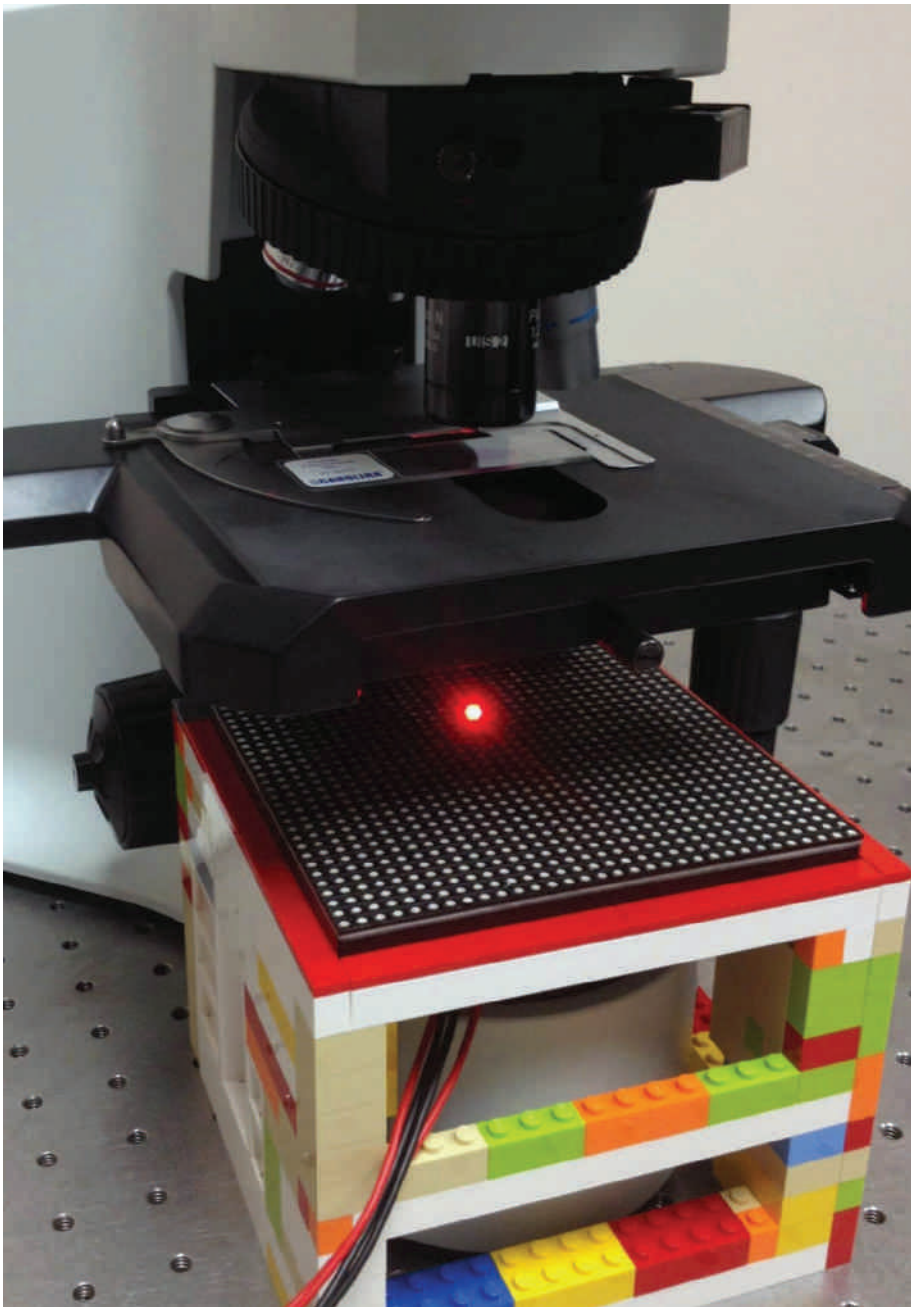


DR GUOAN ZHENG, UNIVERSITY OF CONNECTICUT.

Will machines be able to judge a patient's prognosis? This prototype microscope aims to do part of the job.

SOFTWARE

The computer will see you now

From image-analysis software to lens-free microscopes that fit on a mobile phone, new tools are providing pathologists with clearer and more informative images.

BY KATHERINE BOURZAC

In the seventeenth century, natural historians such as Galileo, Antonie van Leeuwenhoek and Robert Hooke learned to grind lenses and make the first microscopes, revealing the hidden landscapes of life. They saw for the first time the cells in cork, blood and other tissues, and van Leeuwenhoek found swimming 'animalcules' in dental plaque and observed the movement of sperm.

Physicists and engineers are now trying to bring about a similar shift in perspective for microscopy. In most pathology labs, doctors diagnose diseases by poring over tissue slices on glass microscope slides — classifying tumours, for example, based on subtle visual cues that are difficult to quantify. But this is starting to change. Just as lenses once revealed vistas that were previously invisible to the human eye, so software is opening up a new window on biology.

The latest digital tools make it possible to do a visual search in microscopy images, automate diagnosis, and sync image data with the genomic profiles of tumours. Some researchers are even doing away with lenses altogether, creating computational microscopes based on inexpensive hardware that could be used for point-of-care diagnostics, particularly in poor areas with few doctors.

BIG DATA

Pathology has remained stubbornly analogue and qualitative, however. The experienced pathologist's main tools are glass slides, a compound microscope whose design has hardly changed in more than 200 years, and eyes that have seen thousands of tumours. "Most of a pathologist's medical decisions are based on morphology," the structural details of cells and tissues revealed under a microscope, says David Rimm, a pathologist at the Yale School of Medicine in Connecticut.

Just because a method is old is no reason to abandon it, of course. But advocates of digital pathology worry about inconsistencies that can lead to false negatives and misdiagnoses. Experienced pathologists are better than younger ones at identifying rare tumours, but they often disagree with one another and even with their own assessment of the same sample from weeks before.

One hurdle to digitizing clinical microscopy is the size and complexity of the images, says Metin Gurcan, who specializes in biomedical informatics at Ohio State University and was an early advocate of digital pathology. First, a biopsy is sliced into sections and placed on multiple slides. A digital image of a single slide, magnified under the microscope, has about 10 billion pixels and requires about 30 gigabytes of memory. A typical prostate biopsy, for example, uses more than 20 slides and needs about 600 gigabytes.

That's a lot of information for pathologists to scan through — and a lot of data for software to sift. "The number and type of cells found

in these images is mind-boggling," Gurcan says. One way to deal with this complexity is to use software that learns to recognize things in images the same way people do, but faster and more consistently.

VISUAL LEARNERS

Just as people learn by seeing many examples, so can software. In 2011, Harvard Medical School pathologist Andrew Beck built a tool called C-Path (for Computational Pathologist) by feeding learning software with images of breast-cancer biopsies from 248 patients, along with survival data[1]. The software learned to grade the severity of breast cancer and predict patient survival.

A human pathologist who looks at these biopsies under the microscope relies primarily on three features specific to cancer cells to decide how aggressive the tumour is. Do the cell nuclei have an unusual shape? Are the cells dividing? And are the cells connecting with one another as normal, or are they isolated? Pathologists qualitatively score each of these features to determine the tumour grade, a description of how aggressive the tumour is.

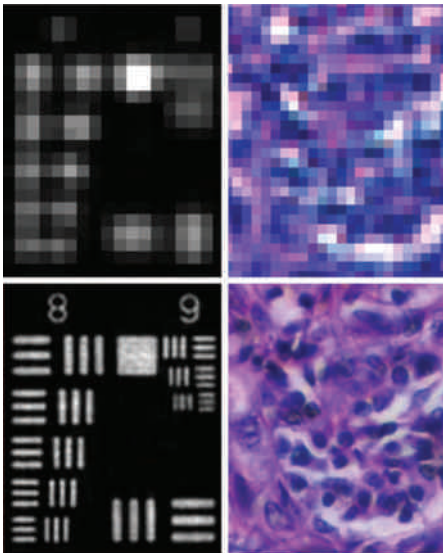
The C-Path system works by segmenting images into small regions called 'superpixels'. It identifies cell nuclei and cytoplasm within each superpixel, and compares the qualities of each superpixel — such as colour, texture, size and shape — with those of its neighbours. For breast cancer, this comparative analysis generates features related to both a sample's global structure and its fine-scale details, such as the average distance between the nuclei of cancer cells and normal cells.

After crunching the training set of images, C-Path came up with 6,642 features, describing not only the tumour cells themselves, which human pathologists focus on, but also the surrounding connective tissue, called the stroma. Indeed, Beck found that the morphology of the stroma was a better predictor of survival than that of the cancer cells alone: an area of stroma that was uniform was associated with a good prognosis, whereas stroma that was infiltrated by epithelial cells indicated more aggressive cancer. Based on its analysis of thousands of features, C-Path was able to predict patient survival more accurately than standard pathological analysis. Beck is now training the software on a broader range of samples, including images of whole slides, and normal breast tissue samples.

It is possible that highly experienced pathologists also look for some of the thousands of features spotted by C-Path but just can't describe them in words. Rimm compares the experience of spotting a tumour with recognizing your uncle in a photo. You can't articulate exactly how you know he's your uncle — is it his nose, eyes, clothing? You just know it's him. But the computer can quantify features in an image, and the analysis is repeatable.

Richard Levenson, a pathologist at the University of California, Davis, says that software such as C-Path has the potential to replace pathologists in assigning grades to tumours. Others believe that the right place for software is as an aide to help physicians navigate large digital images in real time — a second set of very sharp, superhuman eyes. Ulysses Balis, a specialist in pathology informatics at the University of Michigan in Ann Arbor, is developing this latter kind of tool: an all-purpose visual search program called SVIQ.

Balis demonstrates SVIQ with a digital image of a slice of colon adenocarcinoma. He can bring up different fields of the image and zoom in and out. If he finds something interesting — such as a cell that appears to be dividing — and wants to see if there are other similar features in the image, he clicks a 'scan' button and the software highlights all the parts of the image that look similar[2]. In this case, all the dividing cells turn red. The concept is similar to smartphone apps such as Google Goggles or TinEye, which allow a user to upload an image to find out what it is and where you might find more like it. Balis demonstrates the system from his laptop; the image analysis takes about 10 seconds. "This is the simplest possible search structure for a two-dimensional image," he says. "It's a good quick-and-dirty tool."



DR. GUOAN ZHENG, UNIVERSITY OF CONNECTICUT.

Raw data of a ×2 objective lens (top) before being rebuilt as a high-resolution image (bottom).

The SVIQ software can help pathologists find sites of interest in the visually overwhelming landscape of a digital slide. Whereas C-Path is designed to make diagnostic decisions, SVIQ is a way to make digital pathology images more user friendly — and to extract information from them quickly. Jason Hipp, who works on pathology informatics at the National Cancer Institute in Bethesda, Maryland, uses SVIQ to quantify features over an entire slide. Instead of scoring the number of cells that are dividing in a few visual fields, as pathologists do, he says, SVIQ can count every single dividing cell in the entire tissue slice. "When we have to count and measure, it's time consuming," he says. Using SVIQ could speed up the pathologist's work and provide more morphological data.

One of Hipp's research goals is to integrate SVIQ into the process of screening patients for cancer clinical trials. This often starts with a genetic test to find eligible patients. However, for tumours that are dispersed, it can be difficult to find enough cancerous tissue to perform the genetic screen, as the cancer's genetic signal can be lost in the noise from normal cells. It's also difficult to do genetic tests on samples taken by needle biopsies, which are less invasive but produce less tissue to work with, says Hipp. In these cases, pathologists have about 25 minutes after taking a biopsy from the freezer to identify and hand-dye cancerous portions of a tumour slice before it deteriorates. A laser is used to remove the undyed sections, leaving enriched cancer cells for genetic screening.

The SVIQ software can help to digitize this entire process. A pathologist finds an area of the image where there are cancer cells, uses SVIQ to highlight the rest of the cancer, and this map is then sent to the laser cutter. The process takes just 5 minutes.

At the Institute of Cancer Research in London, bioinformatics researcher Yinyin Yuan aims to map all the cell types in a tumour alongside their gene expression data. "The different cell populations in a tumour create a complex landscape that is an obstacle to accurate diagnosis," she says. A sequencing study that samples part of a tumour cannot capture the full picture: it blurs the role played by support cells and misses the heterogeneity of the cancer-cell population. These issues affect patients' prognoses and how they will respond to different kinds of therapy.

In 2012, Yuan developed software to classify the identity and distribution of each of the million or so cells from 300 whole-tumour slides of breast-cancer biopsies, and then integrated this with other '–omics' data. A human pathologist would take too long to go through so many cells in this manner, but a cluster of 100 computing cores, each with the power of a PC, can do the job overnight. The output from Yuan's software[3] is not an image, but data, analysed and collated with other data about the tumour. Yuan found that patients with immune cells that infiltrated the tumour had a better prognosis, and this prediction was strengthened when the image data were coupled with gene expression data. This result is not obvious to the eye of a pathologist staring at a slide and glancing at a list of gene expression data, but it becomes clear when Yuan's software analyses the image. Yuan is now expanding the project to study ovarian and lung cancer.

"What's happening in medical imaging is similar to what happened in astrophysics,"

ANDREW H BECK

Breast cancer image at X200 magnification (left) is broken down into superpixels (black) by an algorithm before it predicts the patient's prognosis.

says Yuan. When astronomers got access to powerful telescopes and digital images, they didn't insist on counting every star — they let computers take over such tedious tasks. It should be the same with digital microscopes and cells, she says.

MATHEMATICAL LENSES

Using software to analyse digital images will make the pathologists' job easier. But it will also lead to new kinds of hardware. Today, creating digital microscopy images means scanning microscopy slides, and this is slow and expensive. Researchers are now building microscopes that can do both jobs, relying more on the power of software and less on lenses and other expensive hardware.

Slide scanners take multiple images, mechanically repositioning the slide under a microscope each time and then stitching the images together. "Mechanical scanning is slow," says Changhuei Yang, who develops microscope technologies at the California Institute of Technology in Pasadena. Because of their expense, these scanners are not typically found in community hospitals. Yang's solution is to increase the field of view and resolution of conventional microscopes. In July 2013, Yang hacked a low-resolution light microscope to create a high-resolution microscope with a wide field of view that can create whole-slide images with cheap hardware[4]. In conventional microscopes, low-power lenses provide a wide field of view at the expense of resolution; high resolution only occurs in small fields of view, hence the need for mechanical scanning. Yang's computational microscope can image an area as large as 120 square millimetres at a resolution of 0.8 micrometres; a comparable standard microscope offers a field of only 1.1 square millimetres at this resolution.

The trick is to use an array of light-emitting diodes programmed to sequentially illuminate the sample with three different colours of light from several different angles; the microscope records a picture each time. These images are then combined, picking apart the way the sample bent or changed the colour of the different light sources, to reconstruct a single, large-area, high-resolution image. Beck, who invented C-Path, says these images are comparable in quality to those made with expensive slide scanners. It's also more efficient, Yang says: "The lead time is shorter, so the number of samples a pathologist can examine can increase." Yang has just started up a company, Clearbridge BioPhotonics, based in Singapore, to commercialize the technology.

Some scientists are going one step further and building totally lens-free microscopes. Aydogan Ozcan, an electrical engineer at the University of California, Los Angeles, is developing microscopes that are basically just light-sensing electronic chips of the kind found in consumer electronics, but altered to cope with wet biological samples. The fancy part is Ozcan's software, which does the same thing as a physical lens: it transforms blurry interference patterns into focused images of cells. His compact microscopes[5] reveal the same details as those with lenses — those made with state-of-the-art chips have a resolution of hundreds of nanometres, clear enough to reveal the nuclei of cells. Sample preparation is similar to that for conventional microscopy.

The components needed to build these microscopes cost just a few dollars, and the calculations can be performed by the processors found in mobile phones. In fact, for demonstration purposes, Ozcan has built several microscopes attached to mobile phones. "We want to empower point-of-care offices or small clinics to work like a hospital lab," says Ozcan.

He is also experimenting with crowdsourcing diagnostics. He uploaded the images of blood cells made by his microscopes to an online game (go.nature.com/mnmsmy) that teaches non-experts to recognize cells infected with malaria. The same images are shown to many different players, and by statistically combining the answers — after removing those clearly trying to upset the system — Ozcan's software generates the same diagnosis as professional pathologists 99% of the time[6]. The idea isn't to 'gamify' pathology — although the games might serve as training tools for medical students and lab technicians. Eventually, Ozcan says, smart software will be able to take over from human pathologists. In the meantime, says Ozcan, "I think we'll see hybrid modalities like this before machine learning takes over completely."

Innovations in computing are set to transform the field of pathology, says Alan Nelson, a physicist and chief executive of VisionGate, a company based in Phoenix, Arizona, that is developing three-dimensional imaging for the automated detection of cancer cells in sputum and blood. "A machine doesn't give an opinion — it can produce data and absolute diagnosis based on statistics," he says. The system could increase screening rates and help patients get the right treatment sooner.

Nelson previously was the lead inventor of the only automated cancer screening test currently on the market. His cervical cancer test, developed at his company NeoPath, received approval from the US Food and Drug Administration in 1996 and is now marketed by Becton Dickinson. This test uses a processor that is custom built for the specific problem of spotting cancer cells in pap smears. The machine is loaded with hundreds of slides that are scanned automatically all day.

Today's computers are capable of much more. Nelson says that microscopes aided by software are now showing biologists and doctors things they've never seen before. Ozcan's lens-free microscopes have revealed new patterns of helical motion in sperm, and VisionGate's three-dimensional images can show pathologists hundreds of previously unseen features. "We can see the texture of the inside of the nuclear surface of a lung cancer cell, and measure the length of the short arm of chromosome six," Nelson says. "My god, it's beautiful!"■

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PERSPECTIVE

The big picture

Many medical images are used once then filed away. This trove of clinical data should be made available to biomedical researchers, says **Alan Moody**.

Whether it is Walmart's one million customer transactions per hour, the 220 billion photos on Facebook, or decoding the human genome's 3 billion base pairs, dealing with vast quantities of data provides an opportunity to extract and exploit previously hidden information. Such is the impact of 'big data' that the World Economic Forum considers it to be a new class of economic asset, comparable to commodities such as gold. The rush to mine this new resource is now underway.

Medical imaging data, which are accumulating in clinics in ever larger amounts, seem to be an ideal target for the 'big data' approach. This information could potentially provide significant insights into health and disease. So far, however, big data — or perhaps more appropriately, the 'big picture' — has not had much impact on this information-rich environment.

WHAT A WASTE

Clinical images tend to be used just once, by a single clinician for a single patient, before being left to gather dust. This represents a squandering of resources. Working cooperatively, whether at the local, regional, national or even international level, researchers and clinicians could use these images to identify trends and correlations, bringing scientific and clinical benefits. But first we need to understand how to harness the current clinical imaging workflow to capture these data.

The move towards using the big picture offers two immediate benefits. First, the image content is self-selecting: patients are being referred for investigation of medical problems, so their images will reflect conditions of clinical relevance and importance. Second, the image data are free of charge, or have already been purchased through current healthcare systems. We could therefore assemble an inexpensive foundation for population-based studies that would otherwise be financially unsupportable.

Consider, for example, the looming medical crisis of dementia. Ideally, the focus will be on identifying at-risk individuals so that prevention and early treatment measures can be deployed. Imaging could hold the key. But in the early stages of dementia the visual clues are likely to be subtle, and identifying them requires investigation of the small subset of the population with the potential to progress. Capturing a sufficient number of appropriate individuals for study would mean casting the imaging net very wide, at immense cost. However, patients with symptoms potentially indicating early dementia — such as vague forgetfulness — may already have been imaged in the course of their clinical visits. What if all the imaging data could be collated? The power of the combined data would allow the small signal contained within the images, denoting early subclinical disease, to rise above the background noise.

Research, as opposed to clinical, image networks are already being built. Examples include the Alzheimer's Disease Neuroimaging Initiative and the Canadian Atherosclerosis Imaging Network. However, these networks are pre-defined by the disease under study; the patients recruited already have overt disease. There remains a need to explore early disease, or pre-disease, at a population level. This can best be achieved by building clinical image networks, allowing recruitment at a far larger scale — a job that will require the repurposing of existing clinical imaging data to create the big picture.

To advance towards this goal, we must change our view of image data. Currently, the development of imaging techniques, image acquisition and analysis, and qualitative interpretation of the image, is done by experts whose aim has been to make their part of the data chain as good as possible — in particular, to produce high-quality images and improve diagnosis. The next, crucial step is to create population-wide image data repositories that are available to researchers.

WE COULD HAVE A POTENT NEW TOOL TO ACCELERATE NOT ONLY BIOMARKER DISCOVERY BUT ALSO THERAPY DEVELOPMENT.

MAKING IT WORK

To exploit this resource, two things are needed. The first is a new breed of image-data scientist. These specialists will be data prospectors searching for a signal arising from the data, viewing combined data rather than individual studies.

The second is a user-friendly network in which to go prospecting. Helpfully, clinical images are now commonly digitized and stored on a picture archiving and communication system, where they can be examined and categorized using a variety of analytical techniques. The biomedical researchers who will use these systems must be closely involved in the design of the processes for storing, managing and accessing the data.

It will be important to embed structured reporting within the big picture. Clinical images from cancer patients, for instance, generate information relating to the primary tumour, lymph-node involvement and metastases. Combining data on a large scale will accelerate insight into primary tumour growth characteristics and associated disease spread.

Ethical standards dictate that the use of such clinical data in a research setting requires consent from each patient, or all-encompassing institutional permission for the pooling of anonymized patient data. Addressing these and other similar requirements will be challenging, but if successful, we could have a potent new tool to accelerate not only biomarker discovery but also therapy development. Institutions that accept these challenges will be seen as pioneers, exploring this new natural resource and potentially reaping the rewards from the wealth of data it contains. ■

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LOMA LINDA UNIVERSITY MEDICAL CENTER, JAMES M. SLATER, M.D. PROTON TREATMENT AND RESEARCH CENTER

Proton computed tomography records the position, direction and energy loss from a proton beam as it traverses a patient's body.

NEXT-GENERATION SCANS

Seeing into the future

From magnetically tagged sugar to smoke-sensing surgical knives and beams of high-energy protons, the next wave of imaging technologies will provide a clearer view of the body.

BY PETER GWYNNE

Medical imaging has advanced rapidly in the early years of the twenty-first century. Doctors can now observe events at the molecular level, examine the characteristics of individual heartbeats, and study processes in the brain in minute detail — tasks all but impossible a decade ago. "We are entering the age of precision medicine," says Roderic Pettigrew, director of the US National Institute of Biomedical Imaging and Bioengineering in Bethesda, Maryland. "We try to be precise in diagnosing, fashioning treatments, targeting treatments, delivering treatments, and monitoring the effects of treatments."

Much of the progress has stemmed from improvements in existing technologies, such as computed tomography (CT), ultrasound and magnetic resonance imaging (MRI). "MRI now allows you to track the diffusion of water molecules in the brain with such precision that you can compute their trajectories along fibre pathways," Pettigrew says.

The latest imaging methods emerging from the laboratory promise to complement these advances. They can detect and monitor cancers, for example, locate individual cells for the delivery of drugs, and provide unprecedented accuracy for surgically treating heart disease and other conditions. Several technologies have yet to reach the preclinical stage, but others have begun the journey to clinical trials. In most cases, they perform tasks currently carried out by conventional systems, but do so faster, more precisely and more safely.

A SPOONFUL OF SUGAR

One such advance in safety relates to a way of identifying tumours. Tumours are avid consumers of glucose, so patients are usually given radioactively labelled analogues of glucose, which congregate in the tumours and are detected by positron emission tomography (PET). But the danger of radioactive exposure prevents this method being used in certain individuals, such as young children and pregnant women, and limits the number of doses for other patients. A team headed by Simon Walker-Samuel at University College London has developed a method that avoids this problem by labelling glucose magnetically with bursts of radio waves instead, so it can be detected by standard MRI. This non-invasive approach is safer — patients merely need to take a sugary drink, rather than a radioactive isotope. It also enables medical teams to differentiate among various types of tumour[1], allowing them both to determine the appropriate therapy more effectively and to assess its effect.

The technique — dubbed glucoCEST, for glucose chemical exchange saturation transfer — measures the exchange of protons between the hydroxyl groups in glucose molecules and water molecules in biological tissue. The pulses of radio waves alter the magnetic character of the protons in the hydroxyl groups, masking the signal from water molecules detected by MRI. "The effect is small, but can be measured if we repeat the experiment a large number of times," says team member Xavier Golay.

The researchers applied the technique to two types of human colorectal tumour transplanted into mice. Studying MRI images taken before they injected glucose into the tumours and one hour after injection clearly revealed differences between the tumour types, as different tumours consistently take up different amounts of glucose. The researchers are now starting human studies: they are recruiting patients with tumours in their neck, and have already scanned about a dozen. For precision, the team injected the glucose into the mouse tumours, but the human patients receive it in the form of a drink. In future, the technology may not be limited to tumours. "One could imagine using it for assessing any organ with a high glucose consumption — for example, the heart or the brain," Golay says.

Other researchers are expressing cautious optimism about the technique. "We feel it is feasible but will require some more MRI development, because the human studies will have to be done at magnetic fields much lower than for the animals," says radiologist Peter van Zijl of Johns Hopkins University in Baltimore,

Maryland, whose team is performing its own human studies of glucoCEST technology.

Another technique, magnetic particle imaging, which was developed in 2001 by scientists at Philips Research in Hamburg, Germany, can potentially provide a faster, more sensitive and safer alternative to the angiography procedures used to assess heart disease, particularly during operations that require simultaneous imaging (see 'The eyes of the operation', page S88). The technique uses magnetic tracers, rather than the chemical contrast agents normally used in angiography — an important benefit because some patients, such as those with chronic kidney disease, cannot safely excrete the standard angiography tracers, iodine and gadolinium.

Magnetic particle imaging relies instead on iron oxide nanoparticles that are injected into the bloodstream. The nanoparticles are superparamagnetic, which means they have an average magnetism of zero but can be magnetized by an external magnetic field — even the weak field generated by a scanner. The process causes the particles to emit small electromagnetic signals that the scanner can detect. Changes in the concentration of the nanoparticles as they sweep through the bloodstream make it possible to monitor such critical factors as blood supply to the heart, the speed of blood flow in the heart, and other data critical to coronary surgery.

So far, researchers have built prototype scanners only suited to small animals, but Steven Conolly, who works on magnetic imaging at the University of California, Berkeley, says the technology has the potential to revolutionize biomedical imaging. Anna Samia, a nanomaterials scientist at Case Western Reserve University in Cleveland, Ohio, says the technology's contrast and sensitivity will exceed those of imaging methods such as MRI, X-rays, ultrasound, PET and CT scans. She adds that it could ultimately be used for the *in vivo* tracking of stem cells and the imaging of inflammation.

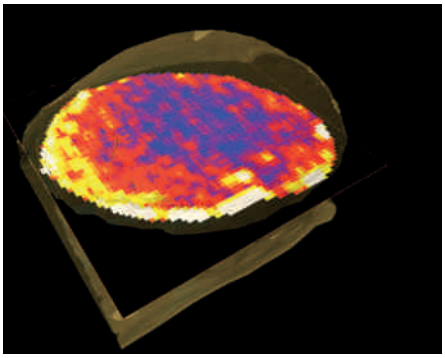
A SMOKING GUN

Working literally at the cutting edge of surgery, a UK–Hungarian collaboration has developed a device that can reveal whether surgeons are slicing through cancerous or normal tissue by sniffing and analysing the smoke it produces[2]. "Modern surgery is based on removing all the tumour," explains team member James Kinross, a surgeon at Imperial College London. "However, there is still no methodology for ensuring this during surgery." Estimates suggest that more than one in five operations intended to remove breast tumours fail to take out all malignant cells.

The 'intelligent knife', or iKnife, uses electrically produced heat to cut the tissue and then analyses the smoke produced using mass spectrometry. It identifies the types and concentrations of the tissue's metabolites and matches the readings to a reference library. This all takes less than 3 seconds, in contrast to the 20–30 minutes needed for histology. As well as accurate results, it also shortens the time a patient spends under anaesthetic.

A group headed by Imperial College's Zoltan Takats and Jeremy Nicholson used the system in the operating theatre to identify in real time 91 pieces of tissue removed from patients against a reference database, without informing the surgeons of the results. In each case, the iKnife's identification of the type of tumour — including tumours from the brain, lung, breast, stomach and liver — matched those determined by traditional methods.

The team is now overseeing an observational trial in three hospitals involving colonic, breast, liver, gynaecological and urological operations. "The next phase will be a randomized trial in which some surgeons will be able to make decisions based on this data and a second group will not," Kinross says. "We will then be able to determine the impact of the device on long-term oncological outcomes." This study may help to address an issue raised by biomedical engineer Nimmi Ramanujam of Duke University in Durham, North Carolina. Many surgeons, she says, would probably prefer to have an image of the tumour's margins before they cut, rather than using the iKnife to feel the edges.



A tumour viewed by glucoCEST: reds indicate higher uptake of glucose compared to blue core.

SIMON WALKER-SAMUEL, UCL CENTRE FOR ADVANCED BIOMEDICAL IMAGING.

BEAM ME UP

One of the existing imaging technologies, CT, uses X-rays, but it doesn't have to. Two approaches under development rely on more exotic forms of radiation: proton beams and synchrotron radiation.

Proton CT records the position, direction and energy loss from a proton beam as it traverses a patient's body. Appropriately treated, the data produce a three-dimensional image of the body that allows physicians to diagnose diseases such as cancer and to plan treatments. George Coutrakon of Northern Illinois University (NIU) in DeKalb explains that using protons instead of X-rays provides a more detailed image of the body's density, because protons release their energy at predictable depths in the body (whereas X-ray energy is emitted in a more continuous manner). This also allows radiologists to target their treatments more precisely, reducing the amount of healthy tissue exposed to radiation.

In 2010, a collaboration between NIU, Loma Linda University in California and the University of California, Santa Cruz, completed a prototype proton-beam imaging system. Now NIU, the Fermi National Accelerator Laboratory and the Argonne National Laboratory are working together to build a second-generation device that can produce three-dimensional images of something the size of a human head in minutes rather than hours.

While these groups are working with protons, an international collaboration is developing a CT imaging system based on synchrotron X-rays. These are produced when charged particles are accelerated around a curved path, and have much higher photon energies than conventionally generated X-rays. Researchers at Ludwig Maximilians University in Munich, Germany, and the University of California, Los Angeles, have applied a novel algorithm to images made by beams at the European Synchrotron Radiation Facility in Grenoble, France. They have produced three-dimensional CT images of the human breast with less radiation exposure than for typical two-dimensional images. Avoiding high exposure to radiation is important because the breast is highly radiosensitive. The method is still in the fairly early stages of research, but the team reports that it could "become a powerful tool for diagnosing breast cancer and allow clinicians to battle the disease more effectively."

Advances in medical imaging don't always happen on such a large scale. Pettigrew cites a recent result from an initiative on low-cost imaging that his institute started nine years ago: the Vscan, a battery-powered, hand-held ultrasound device that GE unveiled in 2009. "Leading cardiologists hold this as the stethoscope of the future," Pettigrew says. "Not only is it smaller and fully portable, it costs 20 times less than the conventional ultrasound device." Under the same initiative, Rebecca Richards-Kortum of Rice University in Houston, Texas, is working with Pentax to develop a microscope small enough to fit into a biopsy needle that can produce real-time diagnoses.

There is so much work underway to develop faster, clearer and safer imaging technologies that some of the benefits may well be coming soon to a clinic near you. "We promote scientists who develop transformative approaches to making new discoveries and acquiring knowledge about the nature of life and physiology and disease," says Pettigrew, speaking on behalf of his institute and the field of biomedical imaging generally. "And we develop new approaches to diagnosing and treating disease." ■

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